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Nanomaterials for Enhanced Photodynamic Therapy

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Abstract

Photodynamic therapy is a non-invasive option for eliminating superficial tumors and to control infections. However, despite some protocols are already approved for the clinic, PDT applications could be much broader if some of its main hindrances were overcome. For instance, the most efficient photosensitizers are hydrophobic, so if one injects them intravenously they tend to aggregate and to be internalized by phagocytes in the blood, impairing the delivery to the target site. In addition, visible light has a limited penetration in tissues, therefore the main applications of PDT are limited to superficial tumors unless an invasive procedure is used for the light to reach deeper sites. Another setback is the hypoxia that commonly happens in tumors, hindering the full potential of PDT as it depends on a constant oxygen supply. In this chapter the reader will find some strategies based on Nanotechnology to overcome these and other obstacles for PDT to reach its full clinical potential, i.e. hypoxia-reverting protocols, X-ray-driven PDT, Cherenkov radiation-driven PDT, and active tumor-targeting.

Keywords: photodynamic therapy, nanotechnology, active targeting, X-PDT, CR-PDT

1. Introduction

Nanotechnology consists on the development of materials with dimensions usually between 1 and 100 nm, where the properties of matter are significantly different than their bulk counterparts, and can be tuned to the desired application. These novel chemical and physical properties are usually derived from quantum effects and from the drastically increased surface-to-volume ratio. Furthermore, since many biological structures, i.e. proteins, organelles, viruses, etc., can be found within the nanometric scale, synthetic nanostructures have easy access to biological systems.

Although Nanotechnology started purely as a physical and materials science, soon the medical properties of nanomaterials became evident, and the new era of nanomedicine and nanopharmacy started. Nanomaterials are now recognized as excellent therapeutic and diagnostic tools, and thousands of novel compounds and nanostructures are developed every year, for the most diverse applications.

As you will see in this chapter, Nanotechnology can help practitioners to overcome several hindrances of photodynamic therapy that have so far prevented this approach from reaching a broader clinical success. Over the last decade, nanostructures have been applied as drug delivery platforms for PDT, and as strategies to enhance the efficiency of photosensitizers in generating ROS upon irradiation.

The nanoparticles can be organic or inorganic, can assume a multitude of shapes and sizes within the nanoscale, can act as photosensitizers themselves or as energy transducers. Even further, nanocarriers prevent the complications that arise from the poor solubility of photosensitizers in aqueous media, and increase the tumor accumulation in order to preserve healthy tissues. We are going to discuss in details the most relevant data regarding the enhancement of PDT by the use of nanomaterials.

2. Nanotechnology in combination with PDT

2.1 Main hindrances of PDT

There is a plethora of photosensitizing compounds available, but the majority of them did not present the requirements for clinical trials, i.e. good solubility, target selectivity, sufficient light absorption on the desired wavelength, and low accumulation in distant sites, especially the skin [1]. One of the biggest hindrances of photosensitizers is their hydrophobicity and consequent tendency to aggregate in aqueous environments, making the intravenous administration difficult unless some kind of delivery system is used [2, 3]. Besides, it is desirable that the photosensitizers accumulate preferentially in the target tissue rather than in healthy sites in order to avoid toxicity, therefore strategies of targeted delivery are often necessary to increase the therapeutic efficiency, and nanoparticle systems offer great advantages in this regard [1].

Besides the tendency to aggregate when photosensitizers are injected intravenously, they tend to be distributed to the whole body in a non-specific way, and to be taken up by plasma proteins or phagocytes, decreasing significantly the efficiency of PDT. To increase the specific delivery and avoid side effects, several carriers have been developed to take advantage of the enhanced permeation and retention (EPR) effect or to actively target tumors and enhance specific accumulation, such as polymer and metal nanoparticles, micelles and liposomes, and magnetic nanoparticles. The EPR effect is caused by the leaky vasculature common to tumor tissues, due to the sinusoid capillaries and the fenestrated endothelial cells, plus the inefficient lymphatic drainage from tumor sites. The active targeting, on the other hand, is actually a plethora of strategies to increase specific tumor accumulation of a drug or therapeutic compound [3].

Another obstacle for photodynamic therapy is the limited penetration of light, so it is used mostly for superficial tumors, or with the help of optic fibers introduced in the patient. Recently, researchers have been developing strategies to produce light inside of the body with the help of nanoparticle scintillators. These materials are able to convert external X-ray photons, which can reach deeper sites in the organism, into visible light photons that could excite a photosensitizer in a process called X-PDT. Another approach to excite photosensitizers with endogenous light is with Cherenkov radiation in a process referred as CR-PDT. Cherenkov radiation is generated when a particle exceeds the speed of light in a defined medium, and is common with the decay of several medical radioactive isotopes [4].

Photosensitizers that absorb in the NIR region, such as indocyanine green (absorption around 800 nm) and aluminum sulfophthalocyanine (absorption around 790 nm), although being able to be used in deeper regions due to the deeper penetration of NIR light, tend to be less efficient in generating singlet oxygen than other photosensitizers that absorb in lower wavelengths. Upconversion nanoparticles (UCNs) can overcome these limitations. The process where the absorption of multiple photons – usually two or three – from a given wavelength leads to the

emission of photons from a shorter wavelength is referred as photon upconversion. This can be used as a strategy to reach deeper tissues with longer wavelengths and excite photosensitizers that absorb in shorter wavelengths and would not be reachable by light otherwise [5].

UCNs usually consist in inorganic luminescent materials, usually made with lanthanide elements that absorb NIR light and emit UV-visible light that can be used to excite more efficient photosensitizers. Other than the possibility of exciting photosensitizers in deeper regions and prevent photobleaching, enhancing PDT efficacy, they can carry a plethora of hydrophobic photosensitizers, either loaded physically or chemically [6, 7].

2.2 Nanomaterials used for PDT enhancement

Nanostructured delivery systems for photosensitizers can provide some major advantages in PDT. The first one is regarding the increased quantity of dyes that can be delivered to the target cell due to the large surface-to-volume ratio, while the second one refers to the prevention of the premature release of the dyes before reaching the target, enhancing the specific accumulation in the target tissue and diminishing the side effects. The third is somehow related to the second, since the loaded dyes find few obstacles in the blood stream and acquire an amphiphilic character once conjugated with nanostructures, enhancing the tumor accumulation as well. Another advantage is the privileged accumulation of nanosized materials in tumor tissues due to the enhanced permeability and retention (EPR) effect. Finally, their surface can be functionalized with a plethora of groups, so that their biodistribution, pharmacokinetics, cell uptake and surface chemistry can be tuned according to the desired application [8]. **Figure 1** summarizes the main advantages of nanotechnology combined with PDT.

Both biodegradable and non-biodegradable nanoparticles can be used to potentiate photodynamic therapy (PDT). In the case of biodegradable nanoparticles

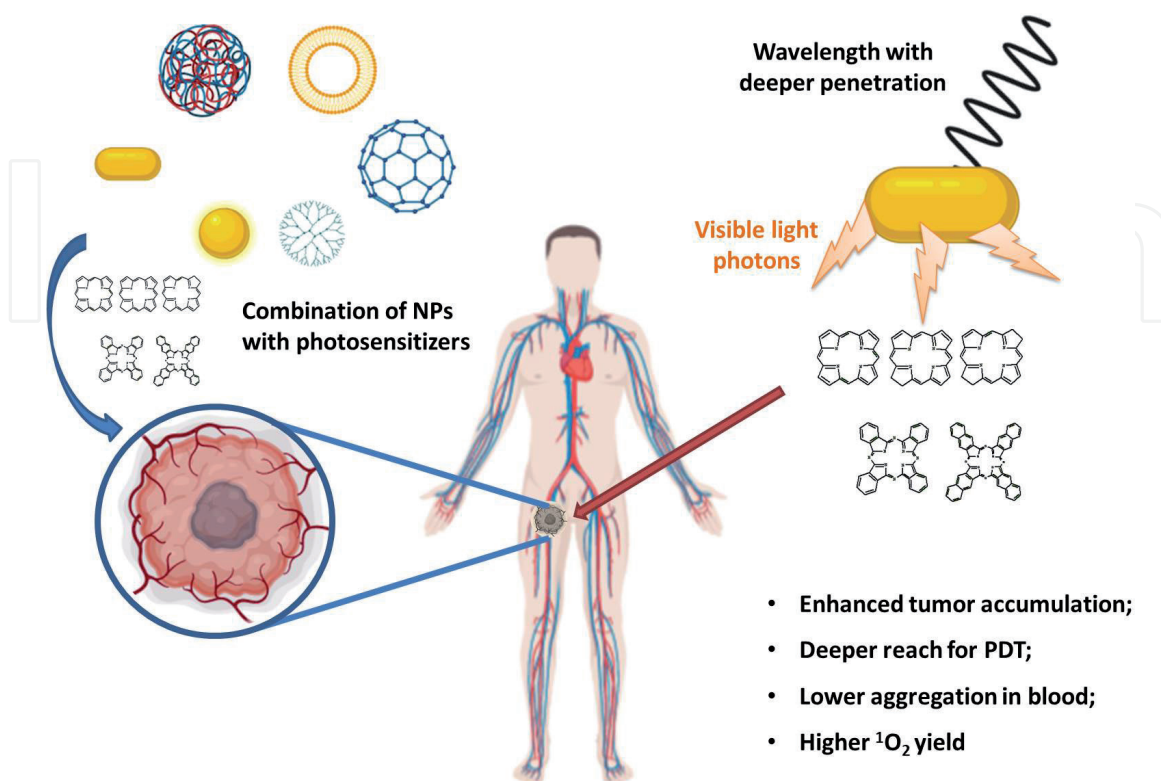


Figure 1.
 The combination of nanoparticles with photosensitizers and its main advantages for tumor ablation.

(generally polymers and lipid-based structures), the photosensitizers are trapped inside them and are released in a controlled manner, so that singlet oxygen can be generated due to the exposition to light. On the other hand, when non-biodegradable nanoparticles are used, usually the photosensitizers are adsorbed on their surface (either external or internal, in case of porous structures), and they do not need to be released completely to generate singlet oxygen [1].

Regarding biodegradable nanoparticles, many photosensitizers have been encapsulated with water-soluble polymers, such as meso-tetra(hydroxyphenyl) porphyrin, bacteriochlorophyll, verteporfin, various phthalocyanines, methylene blue, and hypericin. Their singlet oxygen efficiency depends much on the polymer. Poly lactic glycolic acid (PLGA), for instance, has demonstrated good results compared to other polymers, along with poly lactic acid (PLA) and poly ethylene glycol (PEG). The pharmacodynamics of different polymer nanoparticles may differ from one another, and so do their bioavailability, thus the PDT efficiency may be different according to the nature of the polymer [1].

Non-biodegradable nanoparticles are mostly metallic or ceramic-based (especially silica), but polyacrylamide was also reported as a photosensitizer nanocarrier. Nevertheless, solid silica nanoparticles present higher singlet oxygen yield than polyacrylamide nanoparticles, i.e. 2 to 3-fold more singlet oxygen production by silica nanoparticles loaded with methylene blue compared to their polyacrylamide counterparts [1].

Plasmonic materials, a very important class of non-biodegradable nanomaterials, have proven to act as photosensitizers in the right conditions, and if they have photosensitizers attached to their surface, they can enhance the photodynamic efficiency of the dye. It was observed that semiconductor nanoparticles that present the suitable energy gap can be used as photosensitizers and can also be conjugated with other organic dyes. In these conjugated materials, energy can be transferred from the excited nanoparticles to the photosensitizers through a FRET mechanism [5].

Gold and silver nanoparticles are more stable and present higher extinction coefficients than organic dyes, but if one desires to use them to generate singlet oxygen, O_2 molecules must be adsorbed on their surface in order to provide a rapid energy transfer between the two. Furthermore, the energy transfer from the nanoparticles to the adsorbed oxygen molecules is more efficient in low-energy surface states of metal nanoparticles rather than the high-energy states. When these conditions are fulfilled, it is believed that the PDT antitumor efficiency can be up to 10 times that of chemotherapeutics like doxorubicin [5].

Gold nanorods have been developed by different authors in order to carry phthalocyanines via adsorption onto the nanoparticle surface, either chemically with a thiol group or via electrostatic interaction. The formation of a phthalocyanine layer covering the nanoparticle prevents the aggregation of the hydrophobic photosensitizer and enhances the photodynamic activity [2, 9].

Camerin and collaborators compared the efficacy of a phthalocyanine in its free form and conjugated with gold nanoparticles in ablating B78H1 amelanotic melanoma tumors in mice. The results showed that the accumulation of photosensitizer in the tumor is enhanced when they are bound to the nanoparticles, and as a consequence, the damage was significantly more intense and the tumor growth was significantly slower than the tumors treated with the free phthalocyanines [2].

Several cases of enhancement of the photosensitizer efficiency by plasmonic nanoparticles (due to a strong energy transfer and the prevention of photobleaching) have been reported in the literature, with various photosensitizers and nanoparticle morphologies and materials [9–13]. One interesting example was demonstrated by [14], because the PDT efficiency of the photosensitizer conjugated with gold

nanoparticles was comparable to the free photosensitizer, but the hyperthermal effect contributed to a more intense cytotoxicity against the tumor cells.

The material and the morphology of the plasmonic nanoparticles influence on the extinction coefficients and, consequently, on the energy transfer efficiency. Gold nanourchins, for instance, present an intense extinction coefficient at 940 nm, which is within the therapeutic window, and the singlet oxygen production was intense and sufficed to eliminate cancer HeLa cells while preserving normal NIH-3 T3 fibroblasts. Gold bipyramids can be efficient singlet oxygen generators if the wavelength used overlaps with the surface plasmon resonance peak, even more efficient than methylene blue. Silver and gold nanocubes are unable to generate singlet oxygen, while in the form of nanoprisms the opposite occurs [5].

This dependence on the morphology can be explained by theoretical calculations showing that O₂ can be adsorbed on Au(111), Ag(111), Au(110), Ag(110), Au(100), and Ag(100) surfaces, but on Au(111), Au(100), and Ag(111) surfaces oxygen can remain in molecular form and be excited to its singlet state, whereas on the other surfaces it dissociates into its atomic form. This can only be altered when some defects are present in the crystalline structure [5].

Quantum dots are other promising materials for photodynamic therapy. Graphene quantum dots, for example, reduced tumor cell viability to 20% in a 1.8 μ M concentration, compared to 35% cell viability when the same concentration of PpIX was used. Similar results were obtained for ZnO quantum dots irradiated with blue light [5].

Silica is also widely used as a nanomaterial because it is non-toxic and optically transparent, and their surface chemical functionalization is easily achieved due to the presence of several hydroxyl groups on its surface. When it comes to PDT, silica can act as a carrier of photosensitizers, protecting them from enzymatic degradation and enhancing their permeation in tumors [5]. **Figure 2** shows some of the most important nanomaterials used in combination with PDT.

2.3 Recent advances in X-PDT

X-ray driven PDT makes use of scintillating materials and/or radiosensitizers (**Figure 3**). High-Z elements, for instance, have inner shell electrons which are very efficient in capturing X-ray photons and converting them into relaxed electrons and visible light photons. Thus, the most common scintillators are nanoparticles of high-Z elements doped with rare earth elements, and present

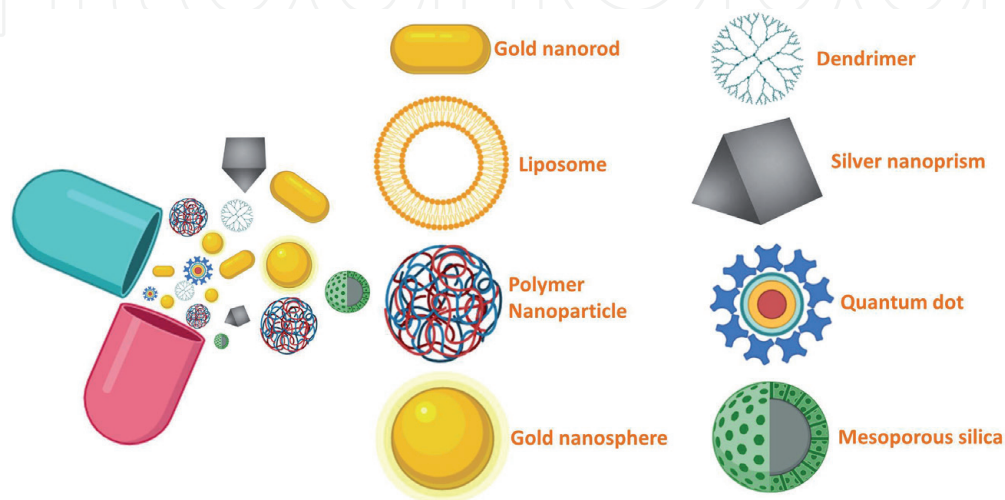


Figure 2.
 Some of the various morphologies and types of nanoparticles used in combination with PDT.

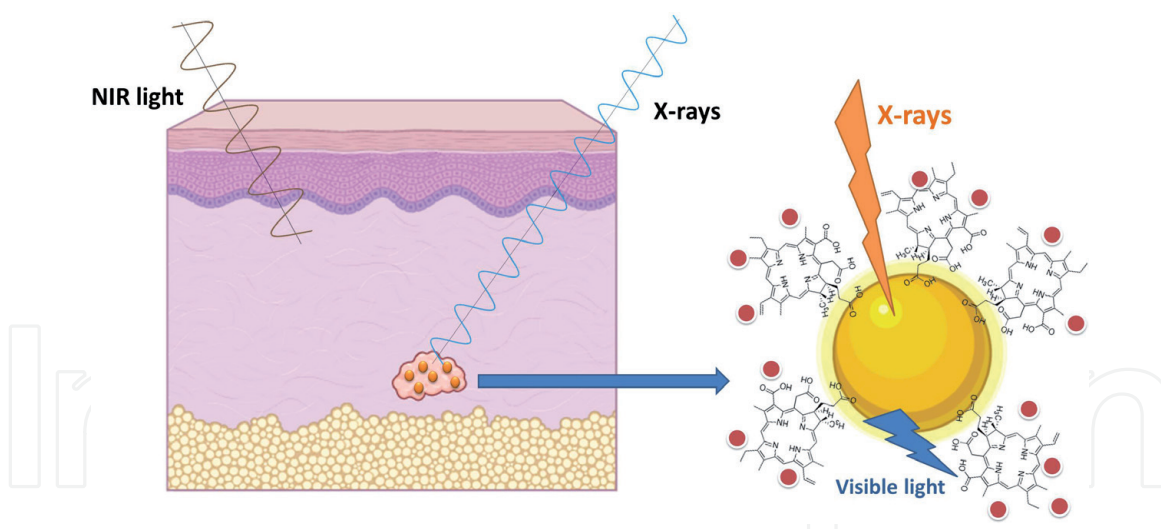


Figure 3.

Deeper penetration of X-rays and its use for X-PDT. The red circles symbolize singlet oxygen generated by the interaction of the excited photosensitizer and molecular oxygen.

useful properties for medical imaging and high-energy physics. The materials can be designed as films, coordination compounds, vitroceraamics, metal-organic frameworks (MOFs), and hybrid organic-inorganic materials, and characteristics such as nanometric size, defects, coatings and media interaction influence on their scintillation properties [4].

Nanoscintillators can be basically divided in doped and semi-conductors scintillators. Lanthanides are the most explored as doped scintillators due to their high density, high-Z, and significant intensity of luminescence, while semiconductor scintillators are mostly composed of porous Si, Si nanocrystals, ZnO, CdSe, CdS, PbS, and CuBr [4].

The efficiency of X-PDT is largely affected by the intensity of X-Ray luminescence, the singlet oxygen yield of the photosensitizer, and the way the photosensitizer is bound to the nanomaterial (either by covalent bonding, electrostatic interactions, or by pore loading). Furthermore, part of the tumor ablation might be due to the generation of UV photons during the scintillation process, apart from the photodynamic effect. The radiosensitization effect must also be considered, since high-Z materials generate ROS whenever their electrons are excited by X-rays into states above the conduction band edge, consequently producing electron hole pairs that interact with water producing hydroxyl radicals, and the electrons generate superoxide and peroxide radicals when they react with O_2 . Those ROS increase the cytotoxicity of the materials under X-ray irradiation [4].

Nanoscale metal-organic frameworks (nMOFs) consist of the self-assembly of metal ions or clusters and bridging ligands, usually organic polydentate. These materials are used as a means to put scintillators and photosensitizers closer to each other, enhancing the singlet oxygen generation efficiency [4].

Nanosized MOFs are usually biodegradable, offering a significant advantage over other nanomaterials, depending on the desired application. They encompass a virtually infinite possibility of structures due to the large availability amount of organic linkers and metallic parts; however, it is of utmost importance to select the components in accordance with the desired application in order to optimize the results. In the medical field, the use of MOFs is still in the prelude, since more pharmacokinetic, pharmacodynamics and biological characterization studies must be performed so that these materials reach clinical trials [15].

Regarding the use of lanthanides as scintillators, Dou et al. synthesized UCNs ($NaYF_4:Yb,Tm$) covalently conjugated with chlorin e6 to prevent the premature

release of the photosensitizer and tested them *in vitro*. The nanoparticles were more efficient than the free photosensitizer even at low concentrations, and the efficacy can be fine-tuned by adjusting the dose of Ce6-UCNs and the laser power [7]. On the other hand, non-lanthanide materials such as SiC/SiO_x core/shell nanowires functionalized with azide groups and porphyrin derivatives were tested for X-PDT and demonstrated significant efficacy. This material showed to be non-cytotoxic in the dark, and emits fluorescence at 545 nm when irradiated with X-rays, exciting the porphyrin derivative in the process [4].

Another example of experimental X-PDT was performed by Sivasubramanian et al. using BaFBr:Eu²⁺ nanoparticles loaded with porphyrins. When irradiated with 3 Gy of X-rays, the nanoparticles generated luminescence that matched the excitation wavelengths of the photosensitizer, leading to photodynamic effect that damaged the DNA, the mitochondria, and generated intense oxidative stress, significantly killing prostate cancer cells *in vitro* [16].

One of the main concerns about X-PDT is the radiation dose that needs to be applied to the patients. In order to diminish the amount of radiation that the patient must be exposed to, some scintillators that present persistent luminescence upon irradiation, rather than fluorescence, are the option. Fluorescence is a phenomenon that lasts for a few nanoseconds, while persistent luminescence can persist for minutes to hours after the excitation, therefore the required dose of radiation for excitation can be significantly decreased. There are evidences that persistent luminescence decreases the rate of oxygen consumption during PDT and may avoid the undesired hypoxia that hinders the photodynamic efficacy [4].

2.4 Recent advances in CR-PDT

Cherenkov radiation-driven PDT, symbolized in **Figure 4**, takes advantage of the fact that most radiopharmaceuticals accumulate in tumors in a selective manner, therefore the photodynamic ablation may occur in a more localized way. However, the generation of Cherenkov radiation occurs in low fluence rates, usually not enough to enable a good photodynamic efficiency [4].

There is a significant advantage, though, of CR-PDT over X-PDT, which is the possibility of targeting multiple metastases easier than with external X-rays.

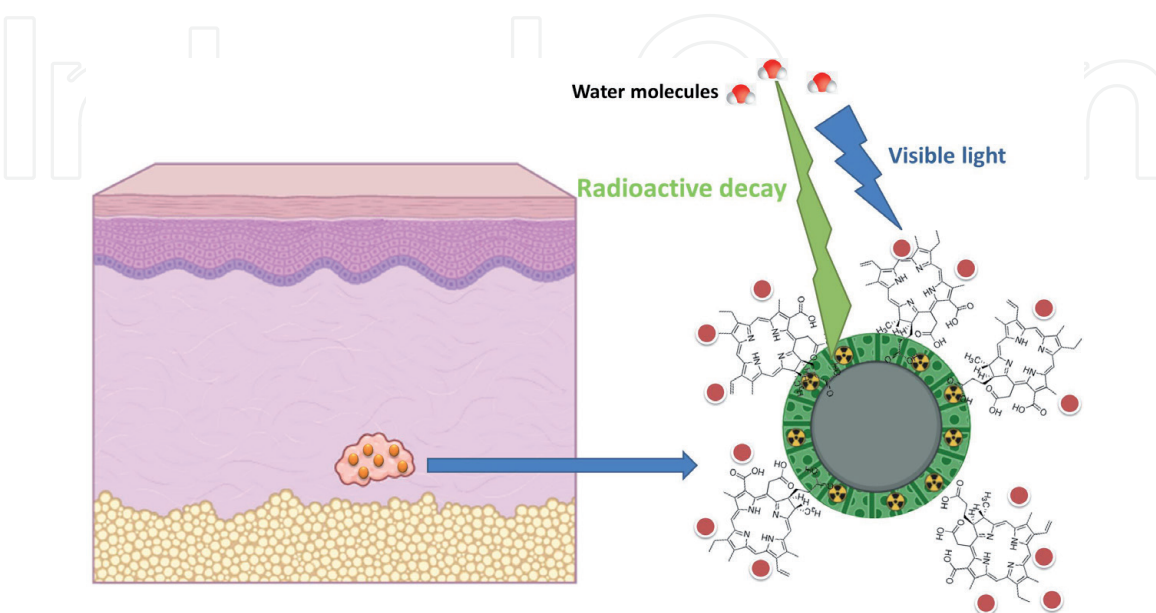


Figure 4. Cherenkov radiation being generated after radionuclide decay, and its ability to excite photosensitizers in order to perform PDT. The red circles symbolize singlet oxygen.

Furthermore, even if the photons generated by the radionuclides are in much lower number than external irradiation (and possibly insufficient to exert significant phototoxicity), it is likely that the damage induced directly by the radionuclides contribute synergistically for the success of tumor ablation with CR-PDT [17].

An example of experimental CR-PDT was performed by Kamkaew et al. The authors encapsulated the radionuclide ^{89}Zr with chlorin e6 into a mesoporous silica nanoparticle. The zirconium Cherenkov radiation emission is mostly in the UV region, but there is a significant emission in the blue region around 400 nm, corresponding to one of the absorption peaks of chlorin e6. The results *in vitro* showed high levels of DNA damage when the photosensitizer is present compared to the radionuclide alone, while *in vivo* results showed complete tumor remission after 14 days, even with a sublethal radiation dose of 15 MBq. However, a significant amount of radioactive nanoparticles were found in the liver after 14 days, so strategies to avoid toxicity to health tissues must be applied [18].

Nevertheless, much progress is yet to be made before X-PDT and CR-PDT become official clinic protocols, despite all the successful results that have been obtained so far. The mechanisms of cell death by the combination of radiotherapy and PDT must be fully understood, and the materials used as scintillators must be fully characterized and optimized [4].

2.5 Hypoxia-reverting strategies

PDT efficacy in tumors is limited by the oxygen supply to the tumors, which tends to be reduced due to deteriorated microcirculation, especially in the tumor center. Since PDT consumes oxygen, it increases even further the local hypoxia, preventing the technique to reach its full potential. Therefore, some strategies to increase the availability of oxygen to the tumors while PDT is occurring have been developed in order to increase the tumor ablation [3]. Cheng and co-workers, for instance, loaded photosensitizers that are activated at 780 nm into perfluorocarbon nanodroplets enriched with oxygen with average size of 200 nm. The use of the nanodroplets also increases the half-life of singlet oxygen, so the PDT efficiency is enhanced both *in vitro* and *in vivo*. With intravenous administration, the tumors were significantly ablated, but with intratumor administration the tumors were eliminated completely [19].

It was observed by Kim et al. that O_2 can be efficiently produced via Fenton reaction in cancer tissues due to the abundance of H_2O_2 derived from the tumor metabolism, especially when mesoporous silica nanoparticles are conjugated to manganese ferrite nanoparticles, which are classical Fenton catalysts, and loaded with chlorin e6. This system enabled a continuous PDT process by providing the tissue with the necessary amount of O_2 via Fenton reaction, and could act as a contrast agent for magnetic resonance imaging, acting as a theranostic material [20].

In this regard, cerium oxide nanoparticles provide a good alternative for converting hydrogen peroxide into molecular oxygen and water, even in the absence of light irradiation. They are, therefore, a smart strategy to provide the hypoxic tissues with oxygen to enhance PDT efficacy, as demonstrated by Jia et al. The authors used a mesoporous core-shell structure consisting of $\text{NaGdF}_4:\text{Yb,Tm}@\text{NaGdF}_4$ upconversion nanoparticles coated with CeO_x capable of converting NIR light into UV light, which activates cerium oxide to produce ROS. Since the nanoparticles have a hollow interior, they can also be used as a drug carrier for a combined chemotherapy, besides being very efficient in tumor ablation by PDT [21].

Although most of the oxygen-generating strategies make use of the excess of hydrogen peroxide caused by the intense metabolism of tumors, which can react

with iron cations generating O_2 and hydroxyl radicals. There is a class of materials, however, that uses water as the source of oxygen, the so-called water-splitting materials, commonly used for solving energy and environmental problems. Since water is the major component of the organism, there is a virtually endless supply of oxygen to be used for PDT enhancement. Metal-free C_3N_4 decorated with carbon dots (in order to enhance the water-splitting upon irradiation with red light) was used by Zheng et al. as a water-splitting material. The nanocomposite was conjugated with the compound PpIX-PEG-RGD, consisting of the photosensitizer protoporphyrin IX with polyethylene glycol and the peptide sequence RGD (arginine, glycine, and asparagine) for active tumor targeting and photodynamic therapy. Under 630 nm irradiation, there was an increased O_2 concentration and singlet oxygen production, enabling a significant cell killing without the occurrence of hypoxia [22].

Red blood cells (RBCs) can be used as photosensitizer and oxygen carriers at the same time in order to increase the efficacy of PDT in hypoxic situations. Wang et al., for example, coupled the photosensitizer Rose Bengal and a hypoxic probe on the surface of RBCs. Upon low levels of oxygen, the hypoxic probe can switch to an active state and undergo an orthogonal near-infrared upconversion, resulting in the release of O_2 from the oxygenated hemoglobin when 980 nm light is applied. The photodynamic process is, thus, kept for longer and results in a better tumor ablation [3].

On the opposite side of the previous strategies, a protocol has been developed in order not to avoid the hypoxia in the tumors, but to use it to potentiate chemotherapy after PDT has been performed. This is possible with the use of hypoxia-activated prodrugs such as triapazamine or apaziquone. He and collaborators used nanoscale metal-organic frameworks (NMOFs) as porous nanocarriers of photosensitizers and hypoxia-activated chemotherapeutics. Both *in vitro* and *in vivo* results indicate an on-demand release behavior of the nanoparticles and an intense tumor ablation, therefore it consists on a promising antitumor strategy [23].

2.6 Tumor-targeting and specific delivery strategies for PDT using nanomaterials

One of the most common strategies of actively targeting specific organs or tissues is by the use of antibodies. Stuchinskaya and collaborators combined the versatility of gold nanoparticles with a hydrophobic photosensitizer (zinc phthalocyanine derivative), preventing its aggregation before reaching the target, and decorated the nanoparticle with tumor-specific antibodies (anti-HER2 for breast cancer) by covalent bonds formed with the coating layer of polyethylene glycol. There was a high efficiency in singlet oxygen generation in cancer cells after a selective targeting [24].

Active targeting can also make use of membrane proteins that are overexpressed in tumor cells, i.e. some integrins and neuropilin-1. By coupling ligands like RGD (a tripeptide composed of arginine, glycine, and aspartate), biotin, and folic acid to nanocarriers, the tumor accumulation is significantly enhanced [3]. Organelle targeting is also an option, especially when it comes to mitochondria. Several lines of evidence show that targeting the mitochondria for PDT avoids drug-resistance by tumor cells via a decreased level of intracellular ATP (the drug resistance phenotype in tumor cells is often associated with overexpressed ATP-driven transmembrane efflux pumps), besides the fact that damage to the mitochondria often leads to cell death [25]. Targeting the lysosomes can be additionally useful because the leakage of protons and hydrolases into the cytoplasm can damage inner structures and lead to cell death [3].

Another reason that makes organelle-targeting important is the short action range of singlet oxygen (no more than 20 nm), so a localized photosensitizer excitation is required. Hou et al. developed a $\text{Fe}_3\text{O}_4@\text{Dex-TPP}$ nanoparticles that enhance the oxygen concentration in tumor cells via Fenton reaction, target the mitochondria (via the triphenylphosphine group, TPP), and are able to be imaged by magnetic resonance imaging due to the magnetic behavior of Fe_3O_4 . This system was loaded with the photosensitizer protoporphyrin IX and grafted with a reduced glutathione-responsive moiety. Upon internalization, Fe^{2+} and Fe^{3+} ions are liberated from the Fe_3O_4 core and diffuse into the cytoplasm, then oxygen is produced by Fenton reaction (Fe^{2+} reacting with the excess of H_2O_2 producing O_2 and hydroxyl radical ($\bullet\text{OH}$)). This allows the PDT process to keep occurring, enhancing the therapeutic efficacy [25].

One of the strategies for specific delivery is the development of pH-sensitive materials that make use of the mild acidity environment found in tumors (around 6.5 to 7.2). Ai and co-workers developed upconversion nanoparticles with a low-pH insertion peptide that in acidic environments allow the insertion of the nanoparticles into the plasma membrane. They observed a large accumulation in the tumor tissue compared to healthy tissues [26].

Calcium phosphate is a biocompatible and biodegradable material, as it is the main component of hard tissues such as bones and teeth. It is sensitive to pH, maintaining its stable structure in physiological pH and dissolves in acidic environments, therefore it can be useful for controlled delivery to tumors. Another advantage relies on the fact that, once inside the cells, calcium phosphate nanoparticles dissolve and liberate calcium ions across lysosomal membranes, impairing the osmotic pressure of the cell and leading it to necrosis [27].

Liu et al. fabricated calcium phosphate-encapsulated core-shell structured nanoparticles ($\text{UCNPs-Ce6}@\text{SiO}_2@\text{Calcium phosphate-Doxorubicin}$), characteristic for being biodegradable, biocompatible, pH-sensitive (which enables the liberation of the chemotherapeutic in the tissue), and provides therapeutic efficiency by PDT upon irradiation with 808 nm due to the presence of Chlorin e6 in its structure. Finally, due to the presence of rare earth elements, it can be used as an imaging tool for diagnostic purposes [27].

Another strategy is the development of nanomaterials that can be degraded by enzymes that are overexpressed in tumors, such as matrix metalloproteinases (MMPs) and hyaluronidase. One good example is the nanomaterial developed by Li et al. [28], which consisted of hyaluronic acid nanoparticles conjugated with chlorin e6 that disassemble in the presence of hyaluronidase and liberate the photosensitizer. This way, they can act as theranostic materials, meaning they can use as diagnostic tools and therapeutic agents. Another example was the MMP2-responsive chimeric peptide nanoparticles coupled with protoporphyrin-IX, which turn from a sphere into large fibers when MMP-2 is present, and this sphere-to-fiber transition contributes to the augmented tumor retention of the nanoparticles [29].

Dai et al. developed a peptide nanoparticle coupled with protoporphyrin-IX ($\text{PpIX-Ahx-K8(DMA)-PLGVR-PEG8}$) responsive to both pH and enzyme. This nanoparticle assumes a spherical shape while in circulation and avoids nonspecific uptake, and when in tumor environments they undergo a charge reversal and cleavage of the PLGVR sequence by MMP-2. Simultaneously, the DMA group is detached because of the low pH. This logic worked to enhance even more the specific uptake by tumor tissues [23].

A very intricate nanosystem combining tumor-targeted PDT with antiangiogenesis therapy and reduced glutathione (GSH) was developed by Min et al.. It consisted on a porphyrinic zirconium-metal-organic framework nanoparticle that

can act simultaneously as a photosensitizer and a carrier of the vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor apatinib. MnO₂ covers the nanoparticle core in order to consume the intratumoral GSH, and the whole system is decorated with a camouflage made of a tumor cell membrane. The tumor specificity was much enhanced, and so was the ablative efficiency of the combined treatment provided by this nanomaterial [30].

In addition to the previous strategies, some researchers have developed nanoparticles that are activated by near-infrared light for selective photodynamic therapy, protecting healthy tissues like the skin. The mechanism of action of these systems is based on the blockage of photodynamic action from the photosensitizer by a co-loaded NIR dye via a fluorescence resonance energy transfer (FRET) effect. The photosensitizer action is recovered once the NIR dye is photobleached by NIR light irradiation in the specific site. Dong et al. developed CaCO₃-PDA-PEG hollow and porous nanoparticles loaded with chlorin e6 for this purpose, and observed that they are degraded in acidic environments such as tumors, liberating the photosensitizer in a selective manner. The generation of singlet oxygen was enhanced in the acidic environment, and the photosensitizer was taken up more efficiently when administered within the nanoparticles, compared to the free photosensitizer and other formulations. It is worthy to mention that when chlorin e6 is injected in a liposomal formulation, the mice present significant weight loss, probably due to an intrinsic toxicity, and this does not happen in the CaCO₃-PDA-PEG formulation [31].

Jeong et al. tested human serum albumin nanoparticles loaded with chlorin e6 in order to develop a more biocompatible system for enhanced PDT efficacy. The nanoparticles, with circa 88 nm in diameter, proved to be non-cytotoxic in the dark, but produced significant amounts of singlet oxygen upon irradiation with the appropriate wavelength. Remarkably, when injected in mice they provided a very specific tumor delivery compared with the free photosensitizer, and simultaneously provided a good imaging property due to the fluorescence of chlorin e6 [32].

PDT can be not only an adjuvant for chemotherapy, but also for immunotherapy, and nanotechnology can potentiate the results and enable the combination of the two therapies in one single approach. That is what was demonstrated by Xu et al. when they developed mesoporous silica nanoparticles made of amorphous silicon dioxide. The nanoparticles were relatively small (around 80 nm in diameter) in order to enhance the cell internalization and avoid side effects, and the pores were large (around 5–10 nm) in order to optimize the drug loading capacity. The nanoparticles were loaded with CpG oligodeoxynucleotide, which is a Toll-like receptor-9 agonist for immunotherapy, and chlorin e6. The authors observed an effective accumulation in tumors in vivo after intravenous injection, and the treatment induced cell damage and the recruitment of dendritic cells. With the immune response elicited, there was a strong cancer vaccination effect, therefore tumors in distant sites can also be affected by the treatment [21].

Finally, a novel phenomenon has been calling the attention of researchers, namely aggregation-induced emission (AIE) of photosensitizers. Some fluorophores are poor light emitters when they are in a single molecule state, but they become strong emitters when aggregated, enabling bioimaging with significant biocompatibility and photostability. Besides, they can generate singlet oxygen in the aggregated state, so they can act as efficient PDT agents. Liu and coworkers synthesized AIEsomes, which are lipid structures conjugated with compounds with AIE property, and tested their efficacy in vivo. Their compounds were biocompatible, provided efficient bioimaging and loading efficiency, ultimately leading to a significant photodynamic effect [33].

3. Conclusion

Photodynamic therapy has long proven to be an efficient way to eliminate tumors and control infections in a non-invasive way. PDT consists on the use of light-absorbent compounds named photosensitizers, which are able to excite O_2 from its ground triplet state to its excited singlet state, or to generate reactive oxygen species whenever they are irradiated with an appropriate wavelength. Much success has been achieved so far, and some PDT protocols are already available for the treatment of tumors, skin infections and for dentistry applications.

Nevertheless, the full clinic potential of PDT is yet to be achieved, mainly due to some limitations of the technique, i.e. the lack solubility of photosensitizers and limited stability in aqueous media such as the blood and the biological tissues (which makes the administration to patients somewhat difficult), the limited penetration of light, especially in the visible spectrum (limiting most of the applications to superficial sites), and the hypoxia that is usually present in tumor tissues, especially the center, and is increased during photodynamic action (since PDT is intrinsically dependent on oxygen, hypoxia hinders the full therapeutic potential of PDT).

Nanotechnology offers potential solutions to these limitations due to the intrinsic properties of nanomaterials, derived mainly from quantum effects that appear in matter in the nanometric scale, and from the surface chemistry that is often optimized in nanomaterials. Nanoparticles can act as photosensitizers given the necessary conditions, or can potentiate the photodynamic properties of attached photosensitizers. Additionally, nanocarriers can be loaded with hydrophobic photosensitizers, avoiding their aggregation and enhancing their specific accumulation in the target site. Finally, upconversion, scintillating and/or radiosensitizing nanomaterials enable the application of PDT in deep-seated tumors because they absorb wavelengths that reach deeper into the organism and emit visible light that can excite photosensitizers in the vicinity.

Nevertheless, some more studies must be performed in order to develop nano-platforms that join the advantages of both Nanotechnology and Photodynamic Therapy, with good biocompatibility and with optimized clinical results. The potential, though, is strong for Nano-PDT to become various protocols for the most diverse medical applications.

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Conflict of interest

The authors declare no conflict of interest.

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